AHP's, HAP's and DAP's:

Modelling potassium current interactions in rat SON magno-cellular neurones

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Introduction

Arginine vasopressin (AVP) and oxytocin (OT) magnocellular neurosecretory cells (MNCs) are neurons that project from the hypothalamus to the pituitary and secrete their hormone directly into the blood.

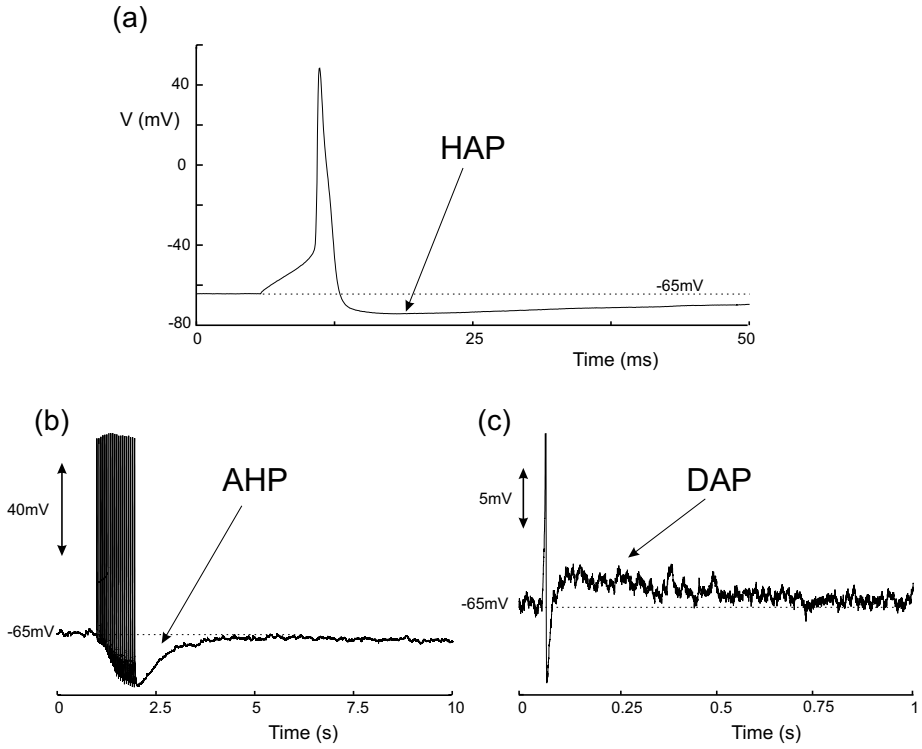
First we first present data from simultaneous calcium fluorescence and electrophysiology and show how electrical activity correlates with calcium elevation, and how spike after-potentials relate to intra-cellular calcium transients.

We then present a mathematical model containing the minimum number of currents and the simplest calcium dynamics needed to reproduce *in vitro* MNC background electrical activity.

After-Potentials

After each spike the membrane potential undergoes several after-potentials before relaxing to rest, ($E_{rest} = -65$mV).

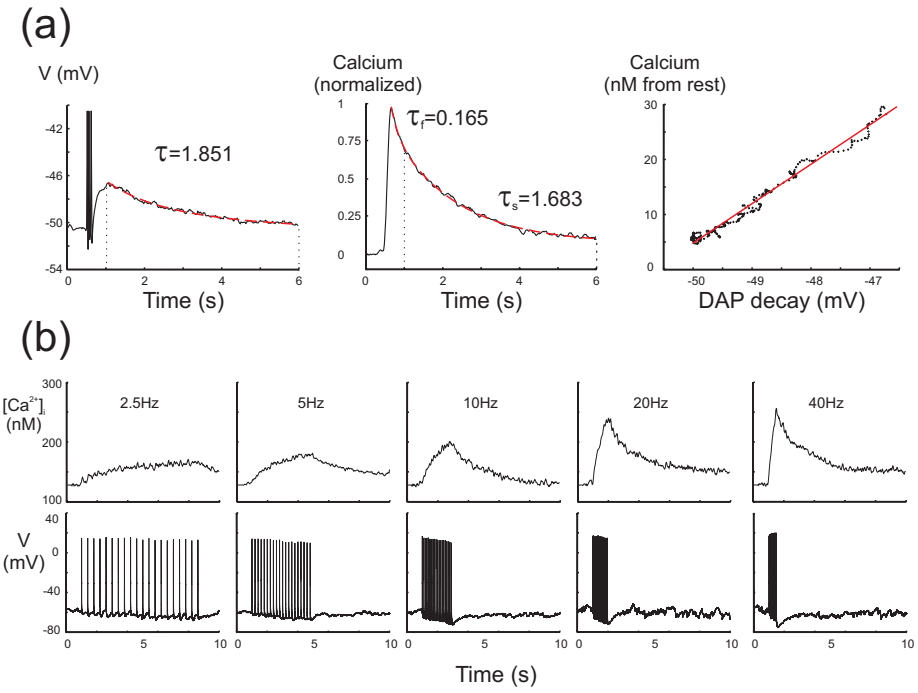
- **HAP**: Repolarization first overshoots E_{rest}, and merges into an ∼8mV, ∼100ms hyperpolarized after-potential.
- **AHP**: a slowly decaying ($\tau = 500$ms) hyperpolarization of maximum amplitude ∼12.5mV is activated by calcium influx during a spike-train.
- **DAP**: a substantial fraction of MNC's express a slow (> 5s) ∼3mV depolarizing overshoot following the initial HAP. The DAP is both calcium- and voltage-dependent.



Spike after-potentials in SON MNC's: (a) the HAP (b) the AHP, and (c) the DAP (note spike has been truncated for clarity). Traces taken from whole cell (a, b) and sharp electrode (c) recordings.

Calcium Concentrations

Simultaneous calcium imaging and electrical recording shows that a DAP can be activated by $\sim$20nM Ca^{2+}, and that its time-course is mono-exponential and follows the clearance of intra-cellular (bulk) calcium $[Ca^{2+}]_i$.

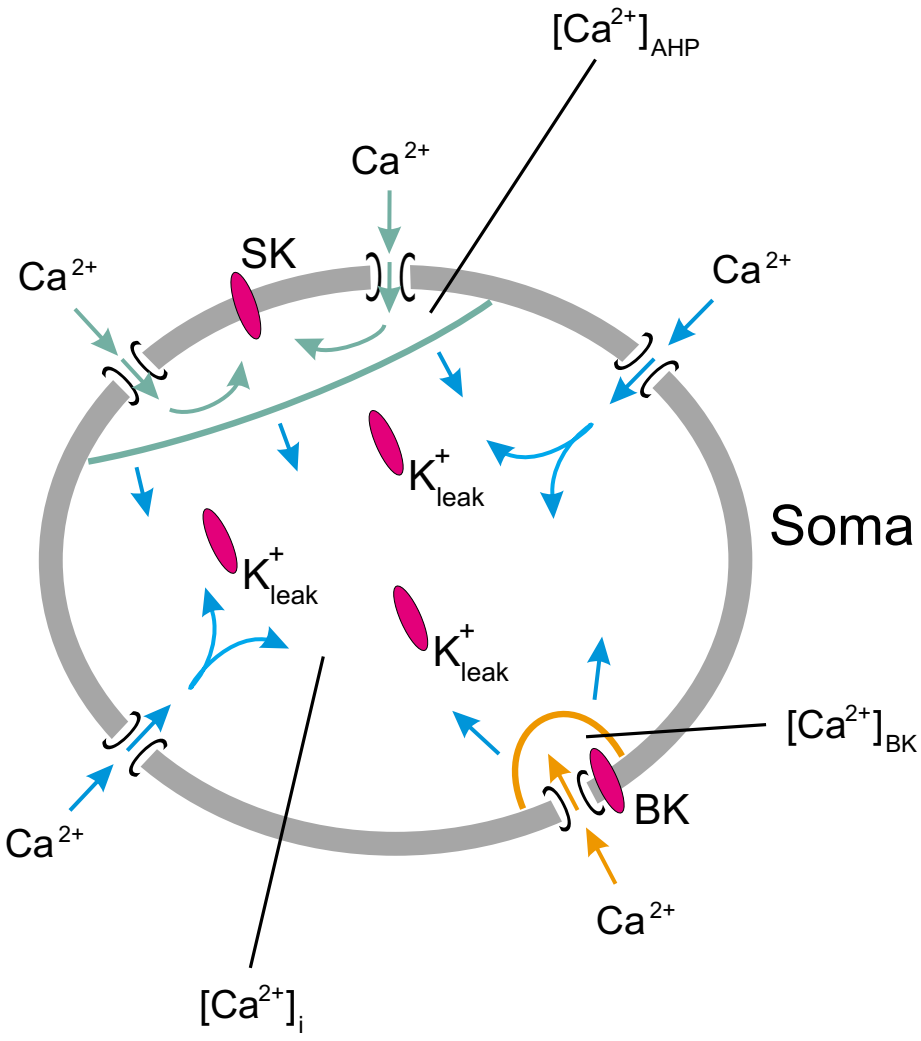


(a) Activation and decay of DAP tracks time-course of $[Ca^{2+}]_i$. Spike truncated for clarity. (b) Influx of Ca^{2+} following trains of spikes, and activation of AHP by $[Ca^{2+}]_i$. Note that AHP decays significantly faster ($\tau = 500$ms) than $[Ca^{2+}]_i$ ($\tau = 2.4$s). Data taken from whole cell recordings from the SON of a hypothalamic slice.

Both calcium rise and clearance for trains of spikes follow a mono-exponential time-course. However rise constant depends upon stimulation frequency, while clearance does not.

Calcium Concentrations

Intra-cellular calcium appears to be compartmentalized. There appear to be (at least) three distinct compartments, each of which activates a single calcium dependent process – I_c, I_{AHP} or $I_{K,leak}$. For simplicity we model this compartmentalization with three distinct pools of calcium, corresponding to local concentrations in three geographically disparate regions. Each is homogeneous and for simplicity we assume that there is no diffusion between them.



Calcium domains and preferential activation of potassium channels. (i) Orange curve indicates Ca^{2+} domain associated with I_C. (ii) Green curve denotes domain associated with I_{AHP}. (iii) The receptors for $I_{K,leak}$ are still further from the site of Ca^{2+} influx and so the current is modulated by bulk calcium (blue) rather than some local transient.

Mathematical Model

We model the electrical activity as a Hodgkin-Huxley type system with a simple calcium dynamics

$$\begin{aligned}
\frac{\mathrm{d}V}{\mathrm{d}t} &= -\frac{I(t)}{C} \\
&= -\frac{1}{C}(I_{Na} + I_K + I_A + I_C + I_{AHP} + I_{Ca} + I_{leak}) \quad \text{(AVP)} \\
&= -\frac{1}{C}(I_{Na} + I_K + I_A + I_C + I_{AHP} + I_{Ca} + I_{SOR} + I_{leak}) \quad \text{(OT)} \\
\frac{\mathrm{d}C_\gamma}{\mathrm{d}t} &= \alpha_\gamma I_{Ca}(t) - \gamma\,(C_\gamma - C_r)
\end{aligned}$$

where γ labels the pool of calcium that activates a single calcium-dependent process, C_γ the concentration in that pool, and $C_r(= 113\text{nM})$ the resting calcium concentration.

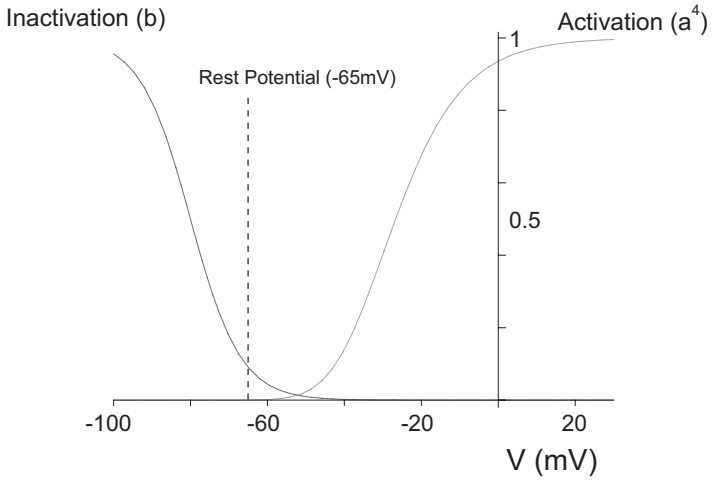
For vasopressin cells we further introduce

$$I_{leak} \equiv G_{leak}(V - V_{rest}) = I_{Na,leak} + I_{K,leak}$$

We have fitted parameters to reproduce the single action potentials and evoked activity that can be observed experimentally in MNC's.

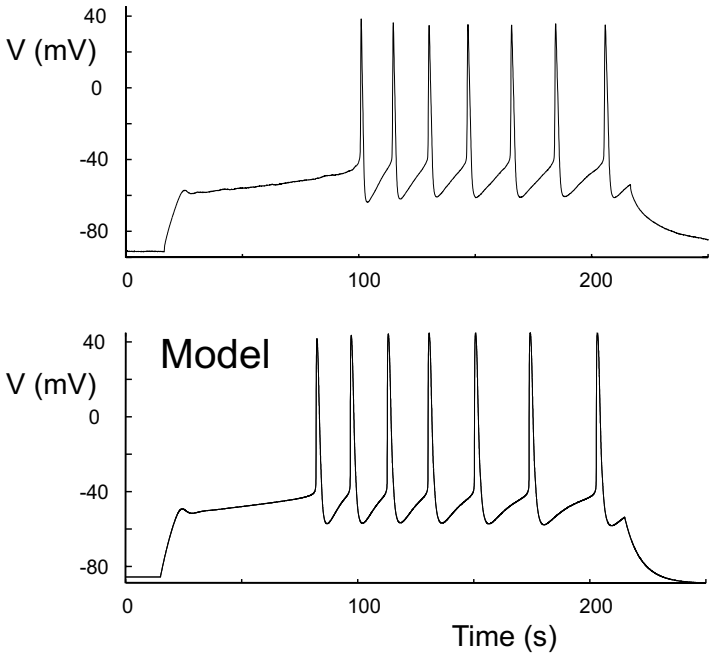
I_A and Spike Latency

The transient outward current, I_A, is significantly inactivated at rest (-65mV) and almost completely inactivated by a depolarization to -60mV.



Activation and inactivation of I_A. The current is significantly inactivated at rest, and completely inactivated by a depolarization of $\sim$10mV. Inactivation can be removed by a conditioning hyperpolarization.

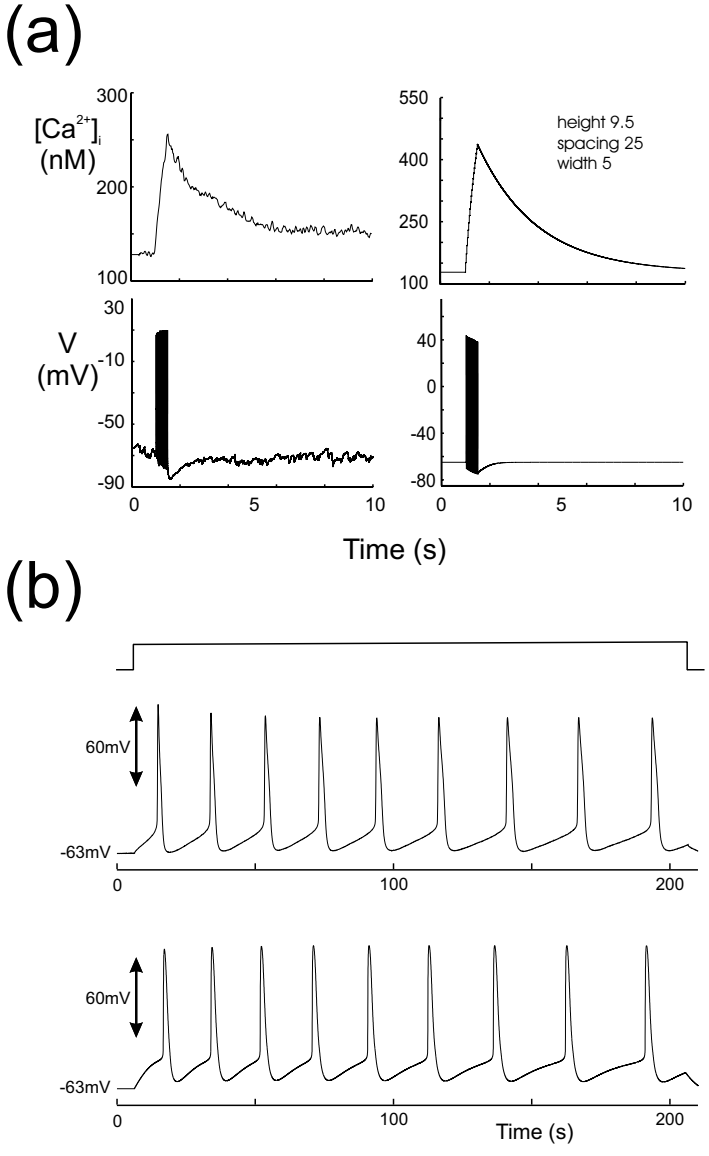
A conditioning hyperpolarization removes inactivation and delays spiking. The rapid activation of I_A appears as a notch on the fast rising phase of the depolarization. Subsequent slow rise is due to progressive inactivation of I_A.



Inactivation of A current and latency to spiking. Note initial rapid rise, followed by notch due to activation of I_A and subsequent slow depolarization as I_A inactivates. The experimental trace (upper figure) is from a whole cell recording from the SON in a hypothalamic slice.

I_{AHP} – the AHP and Spike Frequency Adaptation

Spike trains elicited by long depolarizing current pulses exhibit a progressive increase in their inter-spike interval (spike frequency adaptation - SFA). In addition, a long-lasting net hyperpolarization of the membrane potential, the AHP, is apparent once the spike train has ceased.



(a) Activation of AHP versus calcium increase, both experiment (left) and model (right) stimulated at 40Hz with 5ms pulses. (b) Spike frequency adaptation: top panel experiment, bottom panel model. Note that both cell and model are held depolarized by ~ 3mV to fully inactivate I_A. Experimental data taken from whole cell recordings from the SON in a hypothalamic slice.

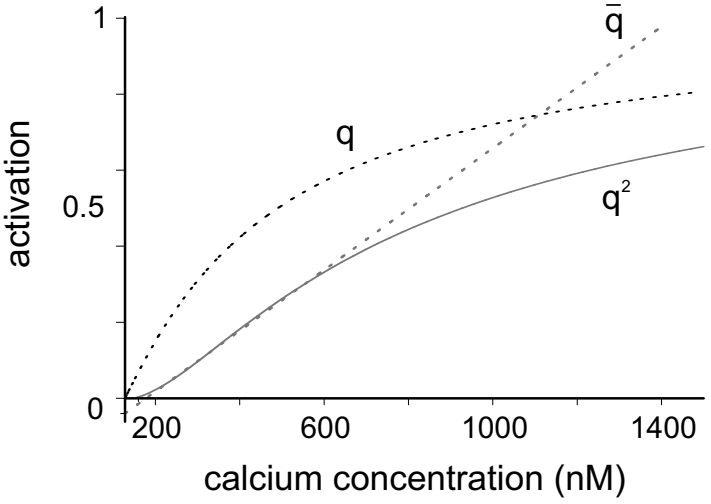
I_{AHP} – Mathematical Model

In other preparations I_{AHP} is described as

$$I_{AHP} \;=\; G_{AHP}\, q_{\infty}^{2}(V - E_K)$$

$$q_{\infty}(C_{SK}) \;=\; \left(1 + \exp\left[-1.120 - 2.508 \log\left(\frac{C_{SK} - C_r}{1000}\right)\right]\right)^{-1}$$

Activation of I_{AHP} as a function of Ca^{2+}. Dashed curve shows q, bold curve shows q^2 and dashed line ($\bar{q}$) is linear fit to q^2. The model indicates first that the AHP does not saturate, and second that q^2 must be kept in the linear region, thus $q_{max}^2 < 0.35$, for mono-exponential decay.



I_{AHP} – the Rise and Fall of the AHP

A progressive activation of the AHP follows each increase of the mean somatic cytosolic (or bulk) calcium ($[\mathrm{Ca}^{2+}]_i$), however decay occurs much more rapidly ($\tau_{AHP} = 400 - 500\mathrm{ms}$ *versus* $\tau_{[\mathrm{Ca}^{2+}]_i} \simeq 2.4\mathrm{s}$).

The AHP decays sharply and within 30ms of cessation of the spike train. Thus q does not saturate, or reach a plateau, during normal activity. If q were to saturate, then the AHP would not decay immediately, but would instead show a lengthy, flat hyperpolarization which would persist until Ca^{2+} decayed beyond the point of saturation.

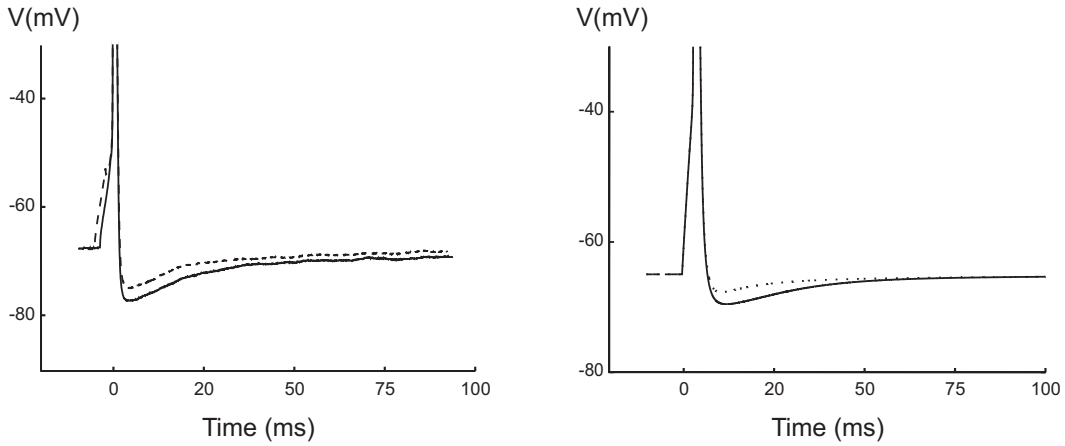
An expression for decay may be found from

$$\frac{\mathrm{d}q}{\mathrm{d}t} = \frac{\mathrm{d}}{\mathrm{d}t} C_{SK} \frac{\mathrm{d}q}{\mathrm{d}C_{SK}}$$

AHP decay can be fit to a single exponential and so if Ca^{2+} decay is also mono-exponential then $\mathrm{d}q/\mathrm{d}C_{SK}$ must remain constant. Thus $q(C_{SK})$ must be linear for most of the decay. Therefore the activation of q_∞ must be confined to the linear portion of the activation curve, and so $0 < q^2 < 0.35$.

I_C and the HAP

Application of 100nM of the BK-specific blocker iberiotoxin (IbTX) slightly increases width at the base of spike by a mean difference of 0.11 ms ($p < 0.02$), but has no effect at half-amplitude ($p < 0.13$). No effects were observed on spike height or rise time. This implies that most of the repolarization phase is carried by other currents (I_A and I_K), and that I_c only becomes active toward the end of the spike.



Effect of pharmacological block of I_c with 100nM IbTX. Left panel shows experiment, right panel shows model. Spike evoked with 3ms depolarizing pulse and hence the initial ramp prior to spiking. Bold line shows control, dashed line shows effect of IbTX. The HAP is still extant when I_c is blocked because I_A and I_K still overshoot the rest potential, but it decays faster and is reduced in amplitude. Experimental data taken from a whole cell recording from the SON in a hypothalamic slice.

Block of I_c decreases the HAP amplitude by $\sim$ 2mV ($p < 0.03$) and decay by 13 ms ($p < 0.006$). When I_c is blocked in the model, the repolarizing currents (I_A and I_K) still overshoot the rest potential and cause an HAP. However, this HAP has a reduced amplitude and also decays faster since it is an inertial effect dictated by the membrane time constant.

I_C – Mathematical Model

Steady-state activation of I_c, $p_\infty(C_{BK}, V)$, is given by [Dopico *et al.*, 1999]:

$$p_\infty = \left[1 + \frac{470}{C_{BK}^{2.38}}\right]^{-1} \left[1 + \exp\left(\frac{(-V - 140\log_{10} C_{BK} + 370)}{7.4}\right)\right]^{-1}$$

However although I_c can be activated by a single spike, its half activation is 470nM Ca^{2+} . Such bulk concentrations are only achieved after prolonged bouts of activity. Thus we propose that each BK channel is activated by calcium in small region close to the channel, that rises and falls rapidly (τ =1ms), and not by bulk calcium.

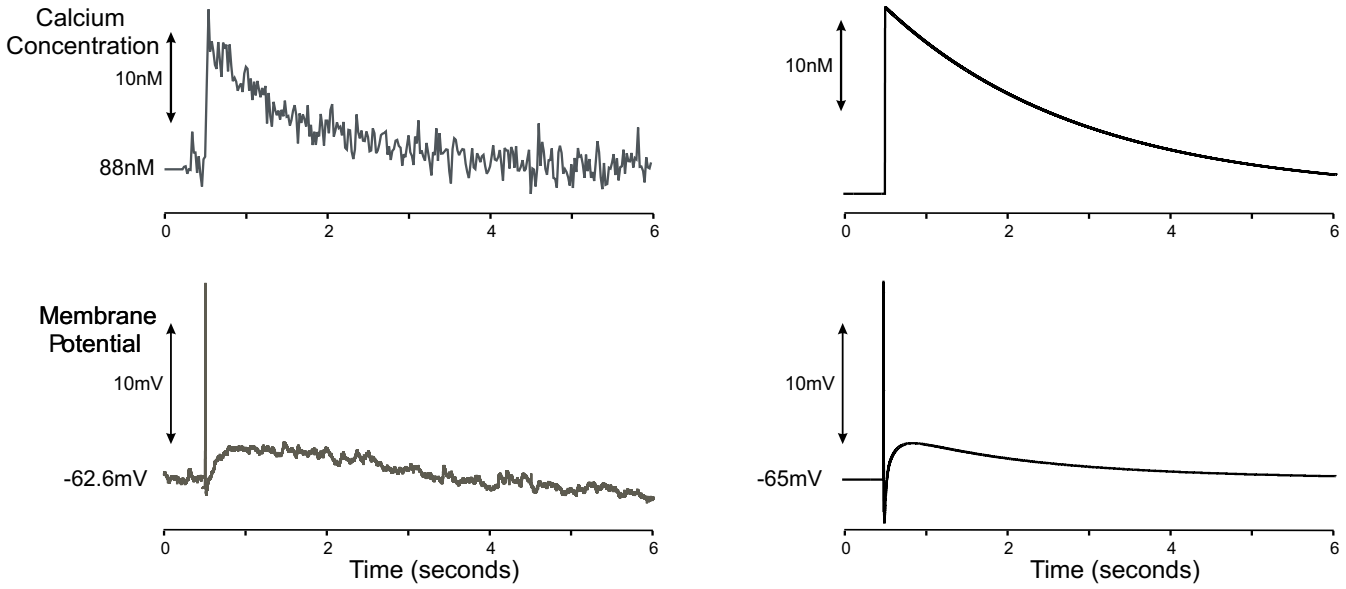
For I_c to be activated during a single spike, activation time constant must be ∼1ms, however de-activation must be much slower to sustain the HAP. We propose that

$$\tau_p = \begin{cases} 1.22\text{ms} & p \text{ activating} \\ 10\text{ms} & p \text{ de-activating} \end{cases}$$

Thus, provided there is sufficient calcium influx, I_c activates towards the end of an action potential. I_c then deactivates slowly once the spike has repolarized and the calcium has been cleared. It therefore outlasts I_A and I_K, keeping the membrane potential hyperpolarized and maintaining the HAP.

$I_{K,leak}$ and the DAP

A depolarized after-potential (DAP) of duration $\sim$ 5s and amplitude $\sim$ 3mV follows each spike. The DAP depends strongly upon calcium and its time course matches $[Ca^{2+}]_i$ decay. Summation of successive DAP's is thought to underly phasic activity when these cells are strongly stimulated.



DAP and associated calcium transient recorded from MNC soma, average of 3 spikes. Electrical activity: whole cell recording from SON hypothalamic explant; Calcium concentration imaged with FURA-2. Spike amplitude has been trimmed for clarity. Modelled DAP and associated calcium transient, spike amplitude has been trimmed for clarity.

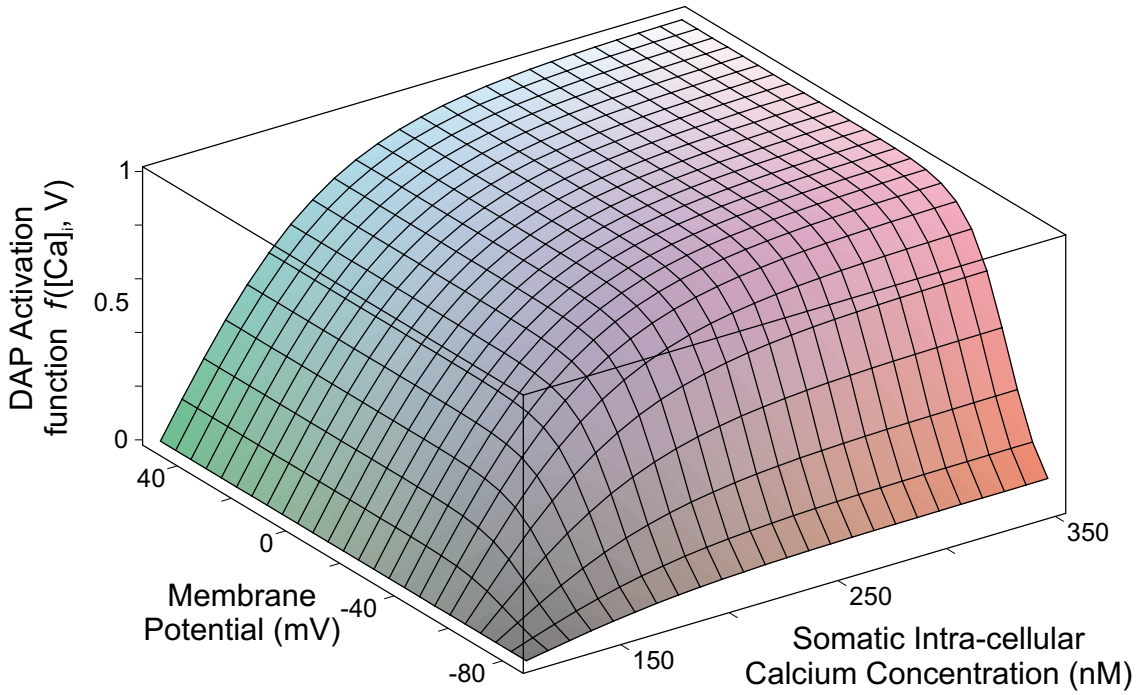
The DAP is thought [Li & Hatton, 1997] to derive from the calcium-mediated inactivation of a resting K^+ channel (*e.g.* TASK-1). We write

$$I_{leak} = I_{Na,leak} + I_{K,leak}$$

We propose that $I_{K,leak}$ has an inactivation function $f([Ca^{2+}]_i, V)$, which can be thought of as an activation function for the DAP ,

$$I_{K,leak} = G_{K,leak}(1 - f)[V - E_K]$$

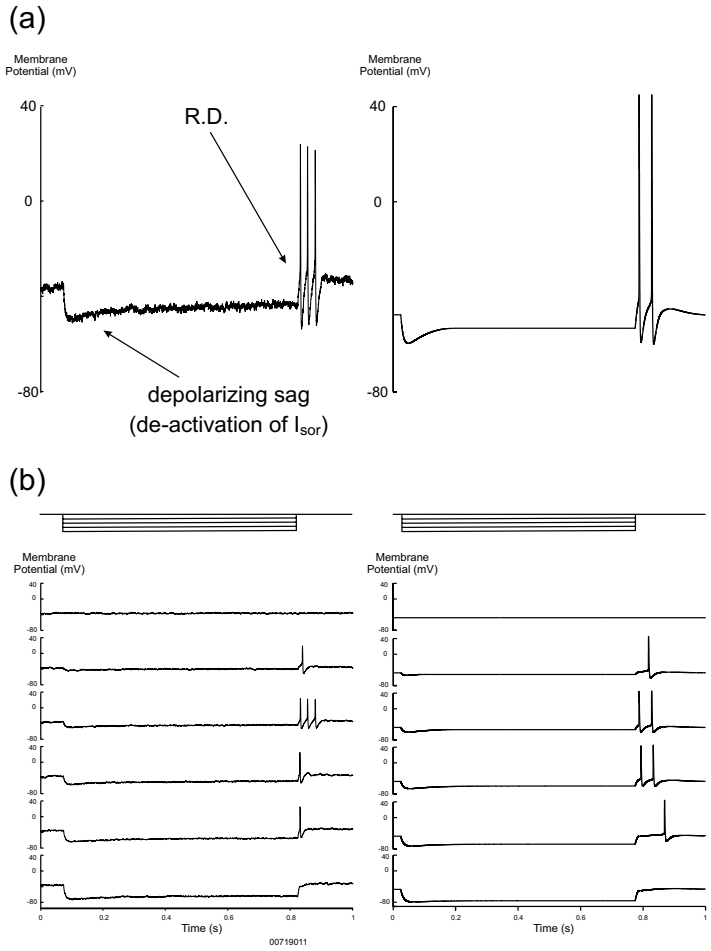
We assume that $f([Ca^{2+}]_i, V)$ has the form:



so that calcium influx from each spike increases f, which inhibits $I_{K,leak}$ and so depolarizes the cell during the time that $[Ca^{2+}]_i$ decays to rest.

I_{SOR} and the Rebound Depolarization

OT cells, but not AVP cells, exhibit a sustained outward rectification (SOR) that becomes active when the cell is held close to threshold.



Sustained outward rectification (SOR) and rebound depolarization (RD), right panel model, left panel experiment. (a) I_{SOR} is activated when V is held close to spike threshold and slowly de-activates when a hyperpolarizing pulse is applied. De-activation causes a depolarizing sag to the voltage trajectory. I_{SOR} remains transiently de-activated when the hyperpolarizing pulse is released and the cell expresses a brief rebound depolarization (RD) which can be large enough to support a short spike train. (b) The magnitude of the RD follows a bell shaped function of the hyperpolarization first increasing as deactivation becomes complete and then decreasing as the A-current de-inactivates. Experimental data is from a sharp electrode recording from an OT neuron in the SON of the hypothalamic explant

Conclusions

- Our SON MNC model incorporates the known *in vitro* properties of these cells and reproduces their firing and their after-potentials.
- We have found a dissociation between the rise and fall of bulk calcium, and the activation of two of the three Ca^{2+} activated K^{+} channels, which we interpret to be due to calcium compartmentalization.
- Calcium has a dual role in these cells – when activating the HAP and AHP it inhibits activity, but when activating the DAP it is excitatory.
- There are duplicate mechanisms for both short- and long-term inhibition in these cells which can be activated either by intrinsic activity (I_c and I_{AHP}) or by extrinsic influences (I_A and I_{SOR}).

Acknowledgements

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References

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Dopico A.M. *et al.* 1999. Rat supraoptic magnocellular neurones show distinct large conductance, Ca^{2+}-activated K^+ channel subtypes in cell bodies versus nerve endings *Journal of Physiology (London)*, **519**(1), 101–114.

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